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Diagnosis of Bacteremia in Whole-Blood Samples by Use of a Commercial Universal 16S rRNA Gene-Based PCR and Sequence Analysis▽

Nele Wellinghausen,[1] Anna-Julia Kochem,[2] Claudia Disqué,[3] Helge Mühl,[3] Susanne Gebert,[1] Juliane Winter,[1] Jens Matten,[4] and Samir G. Sakka[2]*

Institut für Medizinische Mikrobiologie und Hygiene, Universitätsklinikum Ulm, Albert-Einstein-Allee 23, D-89081 Ulm, Germany, and Dr. Gaertner & Partner Laboratories, Ravensburg, Germany[1]; Kliniken der Stadt Köln gGmbH, Krankenhaus Merheim, Ostmerheimerstrasse 200, D-51109 Cologne, Germany[2]; Hochschule Bremerhaven, Institut für Angewandte Mikrobiologie und Biotechnologie, An der Karlstadt 8, D-27568 Bremerhaven, Germany[3]; and MVZ Leverkusen, Abteilung Medizinische Mikrobiologie, Ostmerheimerstrasse 200, 51109 Cologne, Germany[4]

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In a prospective, multicenter study of 342 blood samples from 187 patients with systemic inflammatory response syndrome, sepsis, or neutropenic fever, a new commercial PCR test (SepsiTest; Molzym) was evaluated for rapid diagnosis of bacteremia. The test comprises a universal PCR from the 16S rRNA gene, with subsequent identification of bacteria from positive samples by sequence analysis of amplicons. Compared to blood culture (BC), the diagnostic sensitivity and specificity of the PCR were 87.0 and 85.8%, respectively. Considering the 34 BC-positive patients, 28 were also PCR positive in at least one of the samples, resulting in a patient-related sensitivity of 82.4%. The concordance of PCR and BC for both positive and negative samples was (47 + 247)/342, i.e., 86.0%. In total, 31 patients were PCR/sequencing positive and BC negative, in whom the PCR result was judged as possible or probable to true bacteremia in 25. In conclusion, the PCR approach facilitates the detection of bacteremia in blood samples within a few hours. Despite the indispensability of BC diagnostics, the rapid detection of bacteria by SepsiTest appears to be a valuable tool, allowing earlier pathogen-adapted antimicrobial therapy in critically ill patients.

Bloodstream infection is a life-threatening condition with a high mortality rate, especially in intensive care and neutropenic patients (5, 19, 35, 38). Pathogenic bacteria are the most frequent causes of bloodstream infection, although fungi can also be isolated in a minority of patients (7, 17, 21, 32, 34). Currently, inoculation of blood cultures (BC) is the standard method for microbiological diagnosis of bloodstream infections. However, the limitations of BC include relatively low sensitivities and a long time-to-result for detection and identification of the pathogen, generally over 2 days, and even longer for fastidious organisms (13, 20, 27).

In contrast, DNA-based procedures may offer faster and more reliable diagnoses (3, 30). PCR amplification of microbial genes, followed by detection of amplified products by gel electrophoresis or real-time PCR monitoring using fluorescent dyes or target-directed fluorescent probes, is a quick process allowing pathogen detection within a few hours (18). Identification of microorganisms can be performed by PCR algorithms, taxon-specific oligonucleotide microarrays, or sequencing amplicons (30).

PCR amplification of conserved regions of the bacterial genome, in particular the 16S rRNA gene, combined with sequence analysis is a well-established technique for the identification of bacterial pathogens (18). The main advantages of targeting the 16S rRNA gene are the broad range of pathogens detectable and the independence of this method from the in vitro viability of strains (6). The high sensitivity of detection by PCR of bacterial DNA (15) suggests its use in the diagnosis of bacteremia (16). Initial disadvantages of PCR, notably the incidence of false-positive results from bacterial DNA contaminating PCR reagents (4, 39), have been counteracted by the development of purification methods (12, 28) and the availability of commercial products (22).

We present here a prospective, multicenter study investigating blood samples from patients with suspected bloodstream infections by a new, commercial, universal 16S rRNA gene-based PCR assay (SepsiTest; Molzym, Bremen, Germany) combined with sequence analysis of the amplicon for the identification of pathogens. The results obtained were compared to standard BC diagnostics.

MATERIALS AND METHODS

Study population. The study was performed as a prospective, multicenter study at three hospitals belonging to the University of Ulm, Ulm, Germany, in the Departments of Medicine, Pediatrics, and Surgical Intensive Care between September and November 2008 and the Merheim hospital of the city of Cologne, Germany, in the Department of Operative Intensive Care from January to November 2008. Criteria for inclusion of patients in the study were intensive care unit (ICU) patients with systemic inflammatory response syndrome (SIRS) or sepsis (Ulm and Cologne), hematology/oncology patients with fever (>38.5°C) and neutropenia (neutrophils $< 10^9$ liter^{-1}), or patients with other forms of hereditary or acquired immunodeficiency and fever (Ulm). Within one infectious episode, subsequent samples were collected up to 16 days from the initial sample. The study was approved by the ethics committees of the University of Ulm and the University of Cologne, and informed consent was obtained from all patients

* Corresponding author. Mailing address: Klinik für Anästhesiologie und Operative Intensivmedizin, Lehrstuhl II, Universität Witten/Herdecke, Kliniken der Stadt Köln gGmbH, Krankenhaus Merheim, Ostmerheimerstr. 200, D-51109 Cologne, Germany. Phone: 49-221-8907-3863. Fax: 49-221-8907-8666. E-mail: sakkas@kliniken-koeln.de.
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or parents of patients as appropriate. For ICU patients, informed consent was obtained post hoc.

Blood samples. Blood samples for PCR comprised 9 ml in EDTA from Cologne and 5 ml (adults) or 1.4 to 2.5 ml (children) in citrate from Ulm (Sarstedt, Nümbrecht, Germany). Blood for culture was obtained from the same venipuncture or catheter blood samples. For adults, 20 ml was inoculated into a pair of aerobic and anaerobic BACTEC Plus/F BC bottles (Becton Dickinson, Heidelberg, Germany); for children, 3 to 5 ml of blood was inoculated into BACTEC PED bottles (Becton Dickinson). Samples were sent for PCR from the local laboratory to the central study laboratory in Bremen within 2 days. BC were incubated at the local laboratories in automated BACTEC 9240 systems for up to 7 days. Cultured bacterial isolates were identified by standard microbiological methods.

DNA isolation. As advised by the manufacturer, DNA was extracted and isolated from 1-ml duplicates of whole-blood samples (in children, single 1-ml samples only were drawn) using the SepsiTest kit. The kit includes a protocol for the lysis of human cells and degradation of the released human DNA by a DNase. Pathogenic cells are then concentrated from the blood lysate and treated with a reagent (BugLysis and β-mercaptoethanol) that hydrolyzes the cell walls of any bacteria and yeasts. Pathogenic DNA is then bound, washed, and eluted into 100 μl. Eluates were stored at −20°C until PCR. DNA isolation was performed in a HEPA-filtered hood, decontaminated daily by UV radiation, and strictly separated from PCR processing.

PCR analysis. In addition to the DNA extraction reagents, the SepsiTest kit contains the reagents for the PCR, including Mastermix, DNA-free water for negative controls, DNA for positive controls, and detection reagents for agarose gel electrophoresis. The protocol for PCR and amplicon detection was as advised by the manufacturers. For each set of samples analyzed, negative and positive (*Staphylococcus aureus* DNA, 190 fg/μl; extraction using the PrestoSpin D kit [Molzym]) controls were included. For the detection of amplicons, agarose gel electrophoresis was performed. A sample was considered PCR positive if at least one of the duplicates, if available, was positive. Internal controls (SepsiTest) routinely run with all sample extracts excluded inhibition of the PCRs in all samples.

Sequence analysis. All amplicons (~450 bp) were purified (Qiaquick; Qiagen, Hilden, Germany) and sequenced (GATC, Constance, Germany) using the sequencing primers (SeqGP16, SeqGN16) supplied in the SepsiTest kit. Identification of pathogens was performed by using the online search BLAST tool (1). Genus and species identification was presumed in samples with sequence identities of ≥97 and ≥99%, respectively, to reference sequences of strains in the database. Samples with weak bands in the gel electrophoresis, but no sequencing result were judged as negative.

Interpretation of PCR and clinical data. Patients with positive PCR results but negative BC were classified into patients with "probable to true bacteremia," "possible bacteremia," or "indeterminate PCR result." "Probable to true bacteremia" was assigned if (i) a bacterial species or genus that was detected by PCR was also cultured from a specimen other than blood within 5 days before or after obtaining the blood sample or (ii) the species detected was a typical causative pathogen of the clinical scenario and no other causative pathogen was detected. "Possible bacteremia" was assigned if a species was detected by PCR that represents a common pathogen or known contaminant in BC but was not detected in any other microbiological cultures from that patient. An "indeterminate PCR result" was assigned if a rare or nonpathogenic species was detected by PCR that showed no correlation with other culture results.

Statistical analysis. Calculation of significance was performed by using the Wilcoxon signed rank test. A P value of <0.05 (two tailed) was considered significant.

RESULTS

Study population. The study included 342 blood samples from 187 patients (173 adults and 14 children younger than 18 years), all corresponding to single infectious episodes. Among the study population, 148 patients (79.1%) were ICU patients fulfilling the criteria for SIRS or sepsis and 39 patients (20.9%) were hematological patients with neutropenic fever. Of the 342 samples, BC was positive in 54 samples (15.8%) from 34 patients. Of these 54 samples, 47 were also PCR positive. Among the 288 BC-negative samples, a positive PCR result was obtained in 41.

PCR results in BC-positive samples. Compared to BC samples, the diagnostic sensitivity and specificity of the PCR were 87.0 and 85.8%, respectively. Considering the 34 BC-positive patients, 28 were also PCR positive in at least one of the samples, resulting in a patient-related sensitivity of 82.4%. The concordance of PCR and BC for both positive and negative samples was (47 + 247)/342, i.e., 86.0%. Species and genera detected in the 47 samples by BC and PCR are shown in Table 1. Among the samples, 43 were matched at the species level by BC and PCR, and the others were matched at the genus level (2 *Streptococcus* spp. and 2 *Staphylococcus* spp.). The incubation time of BC until positive detection in the automated BACTEC system was evaluable in 66 BC bottles (Table 1). The mean time to positivity (TTP) was 19.2 h (range, 6.7 to 58.4 h).

The seven samples that were positive by BC but negative by PCR included four BC positive with *Staphylococcus epidermidis*, two with *Escherichia coli*, and one with *Streptococcus oralis* and *E. coli*. In two of the patients with *S. epidermidis* bacteremia (U456 and U480), only one of three and four BC, respectively, revealed a positive BC result after a comparably long incubation time in the BACTEC system (33.7 and 40.9 h, respectively), suggesting a low concentration of *S. epidermidis* in the blood samples (Table 1). However, when we compared the TTP between all BC-positive PCR-positive and BC-positive PCR-negative samples for which the TTP data were available (Table 1), the difference was not statistically significant (for PCR-positive samples, mean TTP = 19.0 h; for PCR-negative samples, mean TTP = 21.2 h [$P = 0.67$]).

PCR results in BC-negative samples. Of the 288 BC-negative samples, positive PCR results were obtained in 41 samples from 31 patients. Accordingly, the resulting detection rate of PCR (25.7%) was higher than BC (15.8%).

In 11 of 12 patients classified as "probable to true bacteremia" (see Materials and Methods), bacterial species identical to the species detected by PCR were cultured from other body sites (Table 2). Among the species identified were *E. coli* (patients U444 and U525), coagulase-negative staphylococci (patients K49, K64, K95, K105, and U507), *S. aureus* (patients K10 and K92), *Klebsiella pneumoniae* (patient K99), and *Raoultella planticola* (patient K44). In patient U516, suffering from pneumonia, *Streptococcus pneumoniae* was detected by PCR.

In a group of 13 patients, classified as "possible bacteremia," bacterial species were detected by PCR that are common pathogens or contaminants (10) in BC but were not found in cultures from other body sites in these patients. Eight of the thirteen patients received broad-spectrum antimicrobials before sampling (Table 2).

In the last group of six patients, classified as "indeterminate PCR result," uncommon or nonpathogenic bacterial species were detected by PCR. These included *Pseudomonas fluorescens* (patient K58), *Aeromonas veronii* (patient U455), and *Petrobacter* spp. (patient K40).

Detection of mixed infections. Polymicrobial growth was observed in four samples from three patients by BC (7.4% [4 of 54 samples]). In three of these four samples, at least one of the causative species was also detected by PCR (Table 3). In the unidirectional sequencing reaction of the SepsiTest kit, two sequencing primers, SeqGN16 and SeqGP16, are used that generally differentiate between gram-negative and gram-positive

TABLE 1. Species spectrum and TTP of positive blood cultures

Patient	Species detected by blood culture	Mean TTP in h (range)[a]		No. positive BC/no. of BC sampled[d]
		Aerobic BC bottle	Anaerobic BC bottle	
Samples with positive PCR result				
K91	*Acinetobacter baumannii*, *Klebsiella pneumoniae*	NA	NA	1/2
U454	*Escherichia coli*	18.9/51.4	20.6/Neg	2/3
U519	*Escherichia coli*	9.1 (8.1–10.9)	11.9 (10.4–14.8)	3/3
K12	*Escherichia coli*	NA	NA	1/1
U473	*Listeria monocytogenes*	14.3 (12.7–15.4)	14.8 (14.1–15.2)	3/3
K16	*Propionibacterium acnes*	NA	NA	1/2
U436	*Staphylococcus aureus* (MRSA)[b]	13.4	15.5	1/3
U477	*Staphylococcus aureus*	17.9 (15.8–19.7)	24.7 (15.8–31.7)	4/4
U515	*Staphylococcus aureus*	40.6	Neg	1/3
U524	*Staphylococcus aureus*	Neg	29.0 (28.0–31.0)	3/3
K104	*Staphylococcus aureus*	NA	NA	1/1
U472	*Staphylococcus capitis*	34.5	Neg	2/3*
	Staphylococcus epidermidis	32.2	58.4	
U461	*Staphylococcus epidermidis*	Neg/Neg/22.4	23.1/18.9/Neg	3/3
U500	*Staphylococcus epidermidis*	19.1	18.4	1/1
U502	*Staphylococcus epidermidis*[c]	11.5 (11.2–11.9)	11.0 (10.4–11.5)	3/3
U517	*Staphylococcus epidermidis*	14.6/15.3	13.1/15.8	2/2
U522	*Staphylococcus epidermidis*	11.9/15.5	10.9/13.9	2/3
K9	*Staphylococcus epidermidis*	NA	NA	1/1
K26	*Staphylococcus epidermidis*	NA	NA	1/1
K47	*Staphylococcus epidermidis*	NA	NA	1/1
K61	*Staphylococcus epidermidis*	NA	NA	1/1
K62	*Staphylococcus epidermidis*	NA	NA	2/2
K65	*Staphylococcus epidermidis*	NA	NA	1/1
K86	*Staphylococcus epidermidis*	NA	NA	1/2
U486	*Staphylococcus haemolyticus*	28.8/34.8	Neg/20.4	2/2
U428	*Streptococcus oralis*	9.4	10.6	2/3*
U465	*Streptococcus oralis*, other alpha-hemolytic streptococci	7.9	8.7	1/2
Samples with negative PCR result				
K17	*Escherichia coli*	NA	NA	1/1
K36	*Escherichia coli*	NA	NA	1/2
U472	*Staphylococcus epidermidis*	Neg	24.7	2/3*
U456	*Staphylococcus epidermidis*	33.7	Neg	1/4
U480	*Staphylococcus epidermidis*	40.2	Neg	1/3
U496	*Staphylococcus epidermidis*	15.9	15.8	1/2
U428	*Streptococcus oralis*, *Escherichia coli*	11.4	6.7	2/3*

[a] NA, not available. Neg, negative.
[b] MRSA, methicillin-resistant *S. aureus*.
[c] The third sample also includes *E. faecalis*.
[d] *, the other positive sample is shown in the lower/upper part of the table.

organisms. By PCR, two bacterial species were detected in 11 samples from nine patients (Table 3). In comparison to the corresponding BC results in BC-positive patients, additionally detected species by PCR mainly included obligate anaerobes, such as *Propionibacterium* and *Veillonella* spp., and species that are frequently found as contaminants. The overall rate of mixed infection detected by PCR was 12.5% (11 of 88 samples).

DISCUSSION

16S rRNA gene-based broad-range PCR is a promising tool for rapid diagnosis of bloodstream infection and several different protocols and systems have been evaluated (9, 11, 23, 25, 29, 31; for a review, see reference 2). Most studies used a general amplification step, followed by hybridization with probes or microarrays to discriminate between higher taxonomic levels or sequencing of the amplicon. The diagnostic performances of these in-house assays varied considerably (for a review, see reference 18). For instance, in a study with 48 newborns admitted to the ICU with suspected sepsis, the PCR detection assay with sequencing of a limited number of samples revealed a diagnostic sensitivity of 66.7% and a specificity of 87.5% (26). In another study, PCR and hybridization probes differentiating between gram-positive and gram-negative taxons to detect bacteremia in 548 blood samples from newborns (14) resulted in a sensitivity and specificity of 96 and 99.4%, respectively. Recently, 16S rRNA gene-based PCR and sequencing of samples from 459 hospitalized patients resulted in a sensitivity and specificity of 74.2 and 99.6%, respectively (25). The heterogeneity of the assay systems with concomitant variability of results and the need for a standardized test for routine use in clinical settings prompted us to evaluate a new commercial system, SepsiTest.

The test comprises a combination of whole-blood sample preparation and universal 16S and 18S rRNA gene detection of bacteria and fungi, respectively. Human blood cells are lysed and the released human DNA degraded, followed by a bind-wash-elute procedure for the isolation of pathogen DNA. The

TABLE 2. Characteristics of blood culture-negative, PCR-positive patients

Patient, age (yr), sex	Underlying disease	No. of PCR-positive samples/total no. of samples	BLAST match (% sequence identity)	Therapy[a]	Other positive cultures[b]
Probable to true bacteremia					
K10, 49, F	Craniocerebral injury, meningitis epidural bleeding	1/1	*Staphylococcus aureus* (99)	Cef (−1), Fos + Met + Cef + Van (+19)	MRSA in CVC, respiratory secretion
K44, 60, F	Amyloidosis, rheumatoid arthritis, pneumonia	1/1	*Raoultella* spp. (98)	Taz (−9), Mer (−5), Cip + Van (−1)	*Raoultella planticola* in CVC, respiratory secretion
K49, 34, F	Gastric resection, pleural effusion	1/2	*Staphylococcus epidermidis* (99)	Imi + Met(−8), Van (−1)	*Staphylococcus epidermidis* in CVC
K64, 66, M	Basal ganglia bleeding	2/3	Sample 1: *Staphylococcus epidermidis* (99); sample 2: *Lactobacillus* spp. (97)	Taz (−1), Van, Cip (0), Imi (+9), Ert (+10), Van (+14), Mer (+25)	*Staphylococcus epidermidis* in tracheal secretion
K92, 84, F	Cerebellar trauma, intracerebral hemorrhage, pneumonia	1/1	*Staphylococcus* spp. (98)	Taz (+1)	*Staphylococcus aureus* in tracheal secretion and nasopharyngeal swab
K95, 70, M	Intracranial hematoma with contusion	1/1	*Staphylococcus epidermidis* (99)	Taz (−4), also Van after CNS result (+1)	CNS in bronchial secretion
K99, 77, F	Meningioma extirpation, cerebral hemorrhage	1/1	*Klebsiella* spp. (98), *Staphylococcus epidermidis* (99)	Taz (−7)	*Klebsiella pneumoniae*, MRSA in nasopharyngeal swab, *Klebsiella pneumoniae* in BC before sampling
K105, 76, M	Hemothorax, multiorgan dysfunction, severe sepsis	1/2	*Staphylococcus epidermidis* (99); *Acinetobacter lwoffii* (100)	Van + Rif + Met + Cap (+6)	CNS in bronchial secretion
U444, 67, M	Rectum anastomosis	3/3	*Escherichia coli* in all samples (100)	Lev (−2)	+ *Escherichia coli* in intraoperative swab (−1), +++ *Escherichia coli* in tracheal aspirate (−2)
U507, 50, M	Subarachnoid bleeding	2/2	Sample 1: *Staphylococcus epidermidis* (100); sample 2: *Pseudomonas fulva* (99)		*Pseudomonas aeruginosa* in CVC (−2), + CNS in thoracic drainage (+5)
U525, 77, F	Ileus	1/1	*Escherichia coli* (100)	Lev (prophylactic), Mer (−1)	+++ *Escherichia coli*, + *Proteus* spp. and + *Enterococcus* spp. in sutural wound (−3)
U516, 53, M	Chronic hepatitis B, pneumonia	1/1	*Streptococcus pneumoniae* (99)	Cef + Cip (−2)	
Possible bacteremia					
K3, 77, M	Basal ganglia and intracranial bleeding, pneumonia	1/1	*Staphylococcus epidermidis* (99), *Clostridium* spp. (97)	Sub + (−2), Cer (+2), Taz (+4), Van (+5)	*Klebsiella oxytoca*, *Candida albicans* in tracheal/bronchial secretion
K28, 59, M	Lung resection, bronchitis	1/2	*Staphylococcus* spp. (97)	Sul + Imi + Cip (−5)	*Pseudomonas aeruginosa*, *Klebsiella pneumoniae* in intraoperative swab and sputum
K55, 49, M	Sepsis of unknown origin	1/1	*Micrococcus* spp. (98)		CNS, *Escherichia coli* in sputum
K63, 58, M	Appendicitis, peritonitis	1/2	*Micrococcus luteus* (99)	Met + Cef (−1), Imi + Van (+1), Cip (+3)	*Enterococcus faecium*, *Enterococcus avium*, *Pseudomonas aeruginosa*, *Enterobacter cloacae*, *Candida albicans* in intraoperative swab, tracheal and bronchial secretion, redon drainage

U446, 47, F	Acute myelogenous leukemia	1/1	*Stenotrophomonas maltophilia* (100)		
U450, 54, M	Chronic lymphatic leukemia	1/1	*Micrococcus* luteus (100)	Imi (−2), Cot + Lev (prophylactic)	
U451, 1, F	Embryonal rhabdomyosarcoma	1/1	*Bacillus* spp. (97)	Cef (after obtaining blood sample)	
U462, 38, F	Acute myelogenous leukemia, peripheral blood stem cell transplantation	1/1	*Pseudomonas aeruginosa* (99)	Lev (prophylactic)	
U464, 62, F	Coronary heart disease, erysipela	1/1	*Pseudomonas* spp.(98)	Aug (after obtaining blood sample), Taz (+1)	
U503, 64, M	Chronic lymphatic leukemia, peripheral blood stem cell transplantation	1/1	*Corynebacterium jeikeium* (99)	Lev (prophylactic)	
U505, 50, F	Acute myelogenous leukemia	1/1	*Corynebacterium* spp. (98)	Taz (−5), Cla (−2)	
U506, 81, F	Endocarditis	1/1	*Streptococcus bovis* (99)	Amp + Gen + Cef (−1)	
U521, 65, F	Endocarditis	1/1	*Propionibacterium acnes* (99)		
Indeterminate PCR result					
K40, 72, M	Peripheral arterial disease	1/2	*Petrobacter* spp. (98)	Cip (+1)	MRSA, *Escherichia coli*, *Enterobacter cloacae*, *Enterobacter faecalis*, *Enterobacter faecium*, *Candida albicans* in intraoperative swab, tracheal secretion
K58, 48, F	Epidermoid of the cerebellopontine angle, meningitis	1/1	*Pseudomonas fluorescens* (99)	Taz (after obtaining blood sample), Taz + Cef + Fos (+24)	*Staphylococcus epidermidis*, *Escherichia coli* in ventricular drainage
K94, 62, M	Incomplete paraplegia, traumatic injury, pneumonia	1/1	*Enterococcus casseliflavus* (100)	Taz (−6), Van + Mer (−5), Cer (−4)	*Raoultella planticola*, *Candida albicans* in tracheal secretion
K101, 64, M	Cerebrocranial trauma	1/1	*Edwardsiella tarda* (99), *Serratia* spp. (98)	Taz (−4)	*Staphylococcus aureus* in tracheal secretion and intraoperative swab
U453, 1, F	Severe combined immunodeficiency	1/1	*Sphingomonas* spp. (98)		
U455, 76, M	Diverticulitis operation	1/1	*Aeromonas veronii* (100)	Imi (−5), Lin (−4)	

[a] The time (in days) of the initiation of antimicrobial therapy or prophylaxis before (–) or after (+) sampling is indicated. Abbreviations: Amp, ampicillin; Aug, augmentin; Cap, caspofungin; Cef, ceftriaxone; Cer, cefuroxime; Cip, ciprofloxacin; Cla, clarithromycin; Ert, ertapenem; Flu, fluconazole; Fos, fosfomycin; Gen, gentamicin; Imi, imipenem-cilastatin; Lev, levofloxacin; Lin, linezolid; Mer, meropenem; Met, metronidazole; Rif, rifampin; Sub, ampicillin/sulbactam; Sul, sultamicillin; Taz, piperacillin-tazobactam; Van, vancomycin.

[b] The time (in days) for the microbiological result of cultures from other material before (–) or after (+) sampling. CVC, central venous catheter; CNS, coagulase-negative staphylococci.

TABLE 3. Patients with detection of mixed infection by BC or PCR

Patient/sample	Organism(s) detected by:	
	BC	PCR[a]
K91/b	*K. pneumoniae*, *A. baumannii*	*K. pneumoniae*
U428/b	*S. oralis*, other alpha-hemolytic streptococci	
U428/c	*S. oralis*, other alpha-hemolytic streptococci, *E. coli*	*Streptococcus* spp., *Leptotrichia* spp.
U465/b	*S. oralis*, other alpha-hemolytic streptococci	*Streptococcus* spp.
K16/a	*P. acnes*	*E. faecalis*, *Propionibacterium* spp.*
K99/a	*K. pneumoniae*	*Klebsiella* spp., *S. epidermidis*
U454/b	*E. coli*	*E. coli*, *Streptococcus* spp.
U454/c	*E. coli*	*E. coli*, *Streptococcus* spp.
U461/d	*S. epidermidis*	*S. epidermidis*, *P. acnes**
U502/c	*S. epidermidis*	*S. epidermidis*, *Veillonella* spp.
K16/b		*S. aureus*, *Propionibacterium* spp.*
K91/a		*S. epidermidis*, *Corynebacterium* spp.
K101/a		*Edwardsiella* spp., *Serratia* spp.
K105/a		*A. lwoffii*, *S. epidermidis*

[a] *, *P. acnes* and other *Propionibacterium* species were identified by sequencing using SeqGN16 (primer for gram-negative organisms).

new sample extraction procedure has recently been used successfully in combination with real-time PCR tests for the detection of pathogens in BC bottles (8) and of *S. aureus*, as well as the staphylococcal *mecA* gene in native blood samples from patients with suspicion of bloodstream infection (37).

In the present study, we analyzed the performance of this system to detect bacteremia in 342 blood samples from 187 critically ill patients. The sensitivity and specificity of the test exceeded 85%. However, the conventional parameters used for the evaluation of new diagnostic tests, such as the sensitivity, specificity, and positive and negative predictive values, do not appropriately reflect the quality and usefulness of a new PCR test, since there is no defined reference for evaluation of the new test. BC has been most widely used as a "gold standard" for statistical evaluation of new PCR tests, and we therefore include these parameters to compare with other studies. However, PCR can detect more cases of bacteremia than culture, since it can detect the DNA of nonviable microorganisms (28). Thus, we also calculated the concordance, both positive and negative, of PCR and BC, which was 86.0%. In general, it appears more appropriate to correlate PCR results not only with BC but also with other microbiological cultures and clinical data from the patients, as shown for PCR-positive but BC-negative patients in Table 2. Among the 88 PCR-positive samples, 41 samples from 31 patients were BC negative. Most of the PCR-positive, BC-negative patients classified as "probable to true bacteremia" or "possible bacteremia" (17 of 25) received antimicrobial substances before blood sampling. This suggests that the bacterial species detected by PCR may have been nonviable and thus not detectable by culture.

In 7 of 54 BC-positive patients the PCR result was negative. This may have been caused by a low concentration of the bacteria in the blood samples, indicated by a long TTP in two patients (Table 1). In addition, in the samples positive for *S. epidermidis*, contamination of the BC during inoculation into the bottles cannot be excluded.

Mixed bloodstream infection occurs in 4 to 8% of patients (18, 24; our unpublished data). In the present study, polymicrobial infections were detected in 7.4% of patients by BC. In a previous study using a multiplex PCR system (33), multiple species were detected in 13% of patients (2 of 15). In the present study using the same sequencing primers, SeqGN16 and SeqGP16, of the SepsiTest kit, a comparable rate (12.5%) was found. Other species found by PCR included mostly obligate anaerobes, which might have failed to grow in the BC, or species that can be regarded as contaminants. Cells of these species might have been present in the blood sample at low concentrations or may have been nonviable in culture. Nevertheless, contamination of the blood sample used for PCR analysis cannot be excluded.

Timely identification of the causative pathogen is important for adequate antimicrobial therapy of septic patients. The SepsiTest can be completed within 4 h, including ~2.5 h of hands-on time for sample preparation and PCR analysis. The test can be performed by any personnel who have been trained to perform molecular diagnostic assays, particularly standard DNA isolation using column-based methods and PCR. The mean time of incubation until positive signaling by the automated BC system was 19.2 h, which is comparable to that reported elsewhere (36). The PCR analysis can be performed within a working day and provided information on the infectious state of patients earlier than almost 70% of the BC in the present study. Definitive identification of detected pathogens by PCR requires, however, sequencing of the amplicon. Taken together, the time spent on amplicon purification, sequencing using modern, fast-cycle sequencing systems, and data analysis, identification can be performed within 3 to 4 h. However, if external sequencing services have to be used, longer turnaround times may make definitive identification of limited use. Nevertheless, in patients with infections caused by fastidious isolates or in cases where blood samples can only be obtained after initiation of antimicrobial therapy, even a next-day PCR result can be valuable.

In conclusion, we evaluated a new commercial test system, SepsiTest, which detects 16S rRNA gene by PCR and identifies any organism by sequencing the PCR product from the whole blood of patients with suspected bloodstream infection. The results obtained by the PCR test correlated well with those from BC. The test exhibited an acceptable rate of presumed false-positive results. Despite the higher costs of molecular diagnostics compared to standard BC (SepsiTest costs 217 U.S. dollars for duplicate analysis of one sample), the PCR test

offers several advantages, notably higher diagnostic sensitivity by detection of nonviable pathogen cells and a shorter total assay time. Thus, SepsiTest should provide a useful supplemental method in the diagnosis of sepsis. Further studies are, however, needed to confirm SepsiTest's usefulness as a diagnostic tool with respect to patient outcome and costs. In addition, the suitability of SepsiTest for detection of pathogenic fungi should be evaluated in further studies.

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